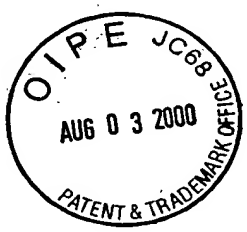
DOCKET NO.: 0660-0139-0XPCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:	:	GROUP ART UNIT: 1641
SHIRLEY LONGACRE-ANDRE ET AL.	:	
SERIAL NO.: 09/125,031	:	Examiner: GRUN
FILED: MARCH 10, 1999	:	
FOR: RECOMBINANT PROTEIN CONTAINING A C-TERMINAL FRAGMENT OF PLASMODIUM MSP-1		



DECLARATION UNDER 37 C.F.R. §1.132

HON. COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C. 20231

SIR:

Now comes Shirley LONGACRE-ANDRE who deposes and states:

1. That I am a graduate of University of California at San Francisco, California, U.S.A. and received my PhD. Degree in the year 1976.

2. That I have been employed by CNRS for 20 years as a Researcher. Attached hereto is my Curriculum Vitae.

3. I am an inventor of 09/125,031 and am familiar with the prosecution history thereof.

4. The following experiments were conducted by me or under my direct supervision and control.

5. The following experiments demonstrate that the native *P. falciparum* has a GC content of 33% whereas a synthetic *P. falciparum* sequence made according to the disclosure in the present application has a GC content of 54%. Furthermore, the following experiments demonstrate that the protein encoded by the synthetic sequence shows significantly more

reactivity with hyperimmune antisera than the native sequence.

6. The nucleotide sequences of *Plasmodium falciparum* p19 as shown in Figure 1A of the present application (reproduced as Figure 1 in the accompanying figures) have a GC content of 33% (native gene-PF19) and 54% (synthetic gene-Bac19).

7. Recombinant baculoviruses were constructed by inserting the native sequence from the Uganda-Palo Alto strain of *P. falciparum* or the synthetic sequence (as described in the present specification) of the C-terminal portion of the Merozoite Surface Protein 1 (MSP 1) of *P. falciparum* from asparagine 1613 to serine 1705. In addition, the baculovirus constructs included sequences coding for Met 1 to Asp 32 from *P. vivax*, which serves as a signal sequence to mediate secretion or surface expression in baculovirus expression systems.

8. Cultures of *Sf*9 insect cells were infected with the recombinant baculovirus containing either the native or the synthetic *P. falciparum* sequences and cultured to allow expression of the sequences. Three days after infection, the infected cells were harvested and the resultant cell lysates separated by electrophoresis on 15% SDS-PAGE gels under reduced or non-reduced conditions. The gels were analyzed by immunoblot with a 1:75 dilution of hyperimmune sera obtained from a pool of 13 antisera derived from *P. falciparum* hyperimmune donors. The results of these experiments are shown in the accompanying Figure 2.

9. The immunoblot data show a lower prominent band migrating in only in those infected cell lysates derived from the synthetic baculovirus contructs (compare lanes 61-64 and 71-74 corresponding to the synthetic gene with lanes P1 and P5 corresponding to the native gene). The results show that cells infected with recombinant virus containing the synthetic *P. falciparum* MSP1 sequence from each of 8 individually isolated baculovirus plaques (Nos. 61-64 and 71-74) exhibited significantly more reactivity with the

hyperimmune antisera than cells infected with 2 different recombinant virus isolates containing the native *P. falciparum* sequence (P1 and P5).

10. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information are believed to be true. Further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

11. Further deponent saith not.

Shirley Longacre-André
Signature

5 July 2000
Date

igure 1. Comparison of the sequences of the artifical gene Bac 19 and the natural *P. falciparum* gene PF 9. Of the 93 *P. falciparum* codons, 36 codons were not changed, the third nucleotide of the codon was hanged in 55 codons and the first and third nucleotides were changed in 2 of the codons. New codons ere added at the beginning to provide the signal sequence and to add an Eco RI site for cloning and two top codons not present in the *P. falciparum* molecule were to terminate the protein. The single letters bove the codons represent the amino acid encoded by the codon; in each case the amino acid for the *P. lciparum* codon is the same as that indicated for the corresponding Bac 19 codon. A star (*) indicates a top codon. The vertical lines indicate the nucleotides that are the same in the two gene sequences.

```
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Bac 19    GAA TTC AAC ATC TCG CAG CAC CAA TGC GTG AAA AAA CAA TGT CCC GAG AAC
                  |||  ||  ||  ||  |||  |||  |||  ||  |||  |||  |||  |||  ||  ||  ||
PF 19             AAC ATT TCA CAA CAC CAA TGC GTA AAA AAA CAA TGT CCA GAA AAT

          S   G   C   F   R   H   L   D   E   R   E   E   C   K   C   L   L
Bac 19    TCT GGC TGT TTC AGA CAC TTG GAC GAG AGA GAG GAG TGT AAA TGT CTG CTG
          |||  ||  |||  |||  |||  ||  ||  ||  ||  |||  ||  ||  |||  |||  |||  |  |
PF 19     TCT GGA TGT TTC AGA CAT TTA GAT GAA AGA GAA GAA TGT AAA TGT TTA TTA

          N   Y   K   Q   E   G   D   K   C   V   E   N   P   N   P   T   C
Bac 19    AAC TAC AAA CAG GAG GGC GAC AAG TGC GTG GAG AAC CCC AAC CCG ACC TGT
          ||  |||  |||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  ||  |||
PF 19     AAT TAC AAA CAA GAA GGT GAT AAA TGT GTT GAA AAT CCA AAT CCT ACT TGT

          N   E   N   N   G   G   C   D   A   D   A   K   C   T   E   E   D
Bac 19    AAC GAG AAC AAC GGC GGC TGT GAC GCA GAC GCC AAA TGC ACC GAG GAG GAC
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          S   G   S   N   G   K   K   I   T   C   E   C   T   K   P   D   S
Bac 19    TCG GGC AGC AAC GGC AAG AAA ATC ACG TGT GAG TGT ACC AAA CCC GAC TCG
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          Y   P   L   F   D   G   I   F   C   S   *   *
Bac 19    TAC CCG CTG TTC GAC GGC ATC TTC TGC AGC TAA TAA
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PF 19     TAT CCA CTT TTC GAT GGT ATT TTC TGC AGT
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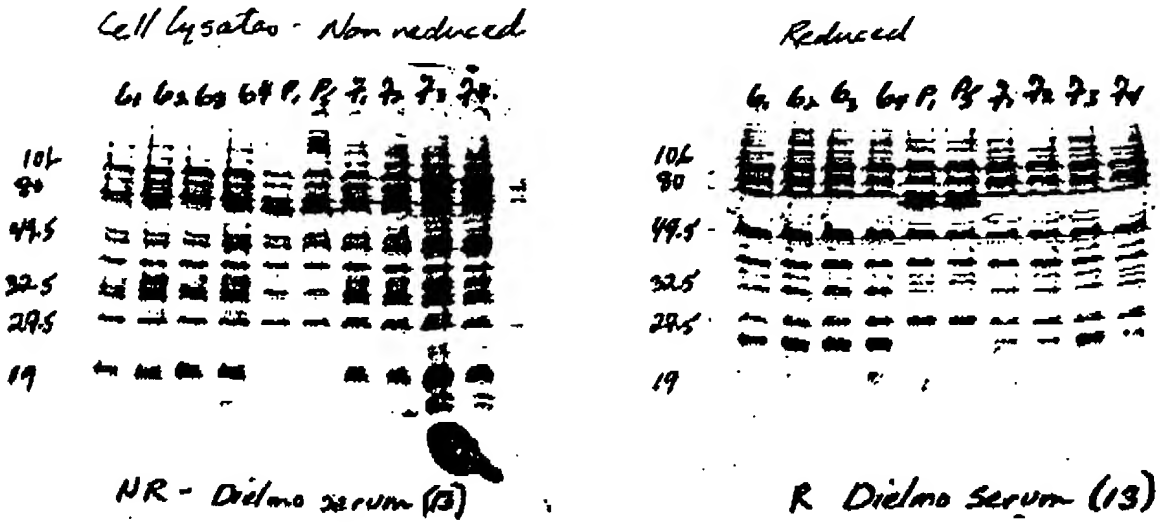
Figure 2. Immunoblots of reduced and non reduced cell lysates infected with recombinant baculovirus. Three days after infection with an excess of recombinant baculovirus the infected cells were harvested, washed with phosphate buffered saline (PBS) and resuspended in PBS and SDS polyacrylamide gel sample buffer with and without 2-mercaptoethanol. The samples were run on 15% SDS-PAGE gels and immunoblotted with a 1:75 dilution of hyperimmune human antisera (pool of 13 donors).



Shirley LONGACRE-ANDRE
Curriculum Vitae

Birth : 2 May 1946 in Washington, D.C., U.S.A.
Nationality : American and French
Married, 4 children
Present address : Unité d'Immunologie des Parasites, URA CNRS 1960
Immunology Department
Pasteur Institute
75724 Paris, France

University Degrees

Bachelor of Arts in Biology, Rice University, Houston, Texas, U.S.A. (1968)
Doctor of Philosophy in Biochemistry, University of California, San Francisco, U.S.A. (1976)

Research Positions

1973-1975 :	Teaching Assistant, University of California, San Francisco
1976-1979 :	Assistante de recherche, Université de Genève
1980-1981 :	Attachée de Recherche au CNRS
1981-1999 :	Chargée de Recherche 1ère classe au CNRS
1999-	Directeur de Recherche 2ème classe au CNRS

Research Laboratories

1969-1970 :	Molecular Biology Institute, University of California, Los Angeles, U.S.A. Director : Paul Boyer
1970-1976 :	Department of Biochemistry, University of California, San Francisco, U.S.A. Director : William J. Rutter
1976-1979 :	Département de Microbiologie, Université de Genève Directeur : Bernard Mach
1979-1984 :	Unité d'Immunoparasitologie, Institut Pasteur, Paris Directeur : Harvey Eisen
1984-1987 :	Unité d'Immunogénétique Cellulaire, Institut Pasteur, Paris Directeur : Jacques Thèze
1989-1995 :	Unité d'Immunoparasitologie, Institut Pasteur, Paris Directeur : Michel Rabinovitch; Luiz Pereira da Silva
1995-1998 :	Unité de Parasitologie Expérimentale, Institut Pasteur, Paris Directeur : Luiz Pereira da Silva
1998-1999 :	Unité de Biologie des Interactions Hôte-Parasite, Institut Pasteur, Paris Directeur : Catherine Breton
1999-	Unité d'Immunologie Moléculaire des Parasites Directeur : Odile Puijalon

PATENT
ATTORNEY DOCKET NO. :0660-0139-OX PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Shirley LONGACRE-ANDRE et al

Serial No. : 09/125,031

Filed : March 10, 1999

For : RECOMBINANT PROTEIN CONTAINING A C-TERMINAL FRAGMENT OF PLASMODIUM MSP-1

Group Art Unit : 1641

Examiner : Grun, J.

DECLARATION REGARDING DEPOSIT OF MICROORGANISMS

I, Madame Danielle Berneman, do hereby declare that:

1. I am a citizen of France.

2. I am the Head of the Department of Patents and Inventions at Institut Pasteur, 25-28 rue du Dr. Roux 75724 Paris Cedex 15 France, to whom this application has been partially assigned. As Head of the Department of Patents and Inventions, I am responsible for protecting the patent rights of Institut Pasteur.

3. Although this application was also assigned to New York University, I was the person responsible for overseeing the deposit of microorganisms.

4. The microorganisms PvMSP1p19A, PvMSP1p19S, PfMSP1p19A, PfMSP1p19S and PcMSP1p19S, which are disclosed on page 30, lines 8 to 17 of this application and the hybridoma G17.2 set forth on page 21, lines 11-14 were deposited as set forth below with the Collection Nationale

de Cultures de Microorganisms ("C.N.C.M.") of Institut Pasteur to assure availability of these microorganisms to the public:

Microorganism	Accession Number	Date of Deposit
PvMSP1p19A	I-1659	February 1, 1996
PvMSP1p19S	I-1660	February 1, 1996
PfMSP1p19A	I-1661	February 1, 1996
PfMSP1p19S	I-1662	February 1, 1996
PcMSP1p19S	I-1663	February 1, 1996
Hybridoma G17-12	I-1846	February 14, 1997

5. The C.N.C.M. has acquired the status of International Depository Authority, within the meaning of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the purposes of Patent Procedure, for deposits of bacteria, bacteria containing plasmids, filamentous fungi, yeasts and viruses. For deposits of microorganisms and other animal cell lines, the C.N.C.M. is a recognized depository institution under Rules 28 and 28a of the Implementing Regulations to the Convention on the Grant of European Patents.

6. The C.N.C.M. has agreed with Institut Pasteur, in consideration of the payment of a lump sum service charge, to maintain the above-mentioned microorganisms and hybridoma and any information thereto and to their deposits in accordance with the Budapest Treaty for 30 years from the date of deposit or for the enforceable life of any patent to issue from this application or for 5 years after the last request for these microorganisms, whichever is longer.

7. All requirements of the C.N.C.M. for maintenance of the above-mentioned microorganisms and hybridoma during the time periods and under the conditions set forth in paragraph 6, above, have been fulfilled by Institut

Pasteur. In this regard, the required lump sum service charge for 30 years maintenance of the above-mentioned microorganisms and hybridoma and for first testing of the viability thereof were paid upfront by Institut Pasteur at the time those microorganisms and hybridoma were deposited.

8. It is believed, therefore, that Institut Pasteur has taken all steps required to have their deposits of the above-mentioned microorganisms and hybridoma maintained by the C.N.C.M. for the following terms: for 30 years or for the enforceable life of any patent after the last request for a sample of cultures, whichever is longer.

9. The C.N.C.M. has also agreed with Institut Pasteur to allow access to the above-mentioned microorganisms and hybridoma during the term set forth in paragraph 8, above, and also during the pendency of this application to anyone determined by the Commissioner to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. § 122.

10. Institut Pasteur has committed itself for a period of at least 30 years to replace at the C.N.C.M. the above-mentioned microorganisms and hybridoma, should they happen to mutate or die, it being understood that any replacement for these microorganisms and hybridoma would have the same features as those already deposited.

11. The C.N.C.M. has agreed with Institut Pasteur to remove irrevocably all restrictions on the availability to the public of the above-mentioned microorganisms and hybridoma upon granting of a patent from this application.

I also declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States

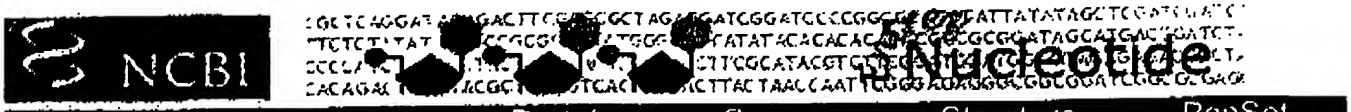
Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

June 26, 2000

Date

Madame Danielle Berneman

Annexe I



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Revised: May 10, 2000.

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1921 aagaatgcca aggtgaagga cgacctgtac gttccaaagg tgtataatac aggcgagaaa
1981 cctgagccct actacttgat ggtcctcaaa agggaaattg acaagttgaa ggacttcatc
2041 cccaaaatcg agagcatgat cgccactgag aaggccaagc cggcagcgtc agcgccagtg
2101 accagtggac aattgcttag aggatcaagc gaagcagcga cagaggtcac aaccaatgcg
2161 gtaacatctg aagatcaaca acaacaacaa caacaacaac aacaacaaca acaacaacaa
2221 cagcaacaac agcaacaaca acaatcacaa gtagtaccag cacctgcagg agatgcccaa
2281 caagtaatct caacacaacc gactagtcaa tccgcagcac caggcgtatc agccacacca
2341 gcaccaacac ctgctgccgc agccgcccca gcaccagcca tgtccaaact ggaatacctc
2401 gaaaagctcc ttgatttttt aaaatccgct tacgcatgtc acaagcacat cttcgtaacc
2461 aactccacca tggacaagaa actactcaaa gagtacgaac ttaacgctga tgagaaaacc
2521 aaaattaatc aaaacaaatg cgatgaattg gacctcctat tcaatgtcca gaacaacttg
2581 ccagccatgt actccatata tgactccatg agcaacgagc tgcagaatct ttacattgag
2641 ctgtaccaga aggaaatggt ttacaatata tataagaaca aggacacgga caagaagatt
2701 aaggctttcc tggaaacatc caacaacaaa gcggctgctc ctgctcagtc agcggcaaaa
2761 cccagcggtc aagcggagta ctactccagt aacgacaact gcgccagtaa ccacaacaac
2821 agttactcca agtccccaaa catcagttgt aacaagcaca cctctacacc ccaagcagaa
2881 gaaaaccaac gcgtgggagg taacagcgag gagaaacccg aagccgacac tgcgcaagtg
2941 gaaaagtttt acgacaagca cctatcccaa attgacaagt acaacgatta tttcaagaaa
3001 ttccttgaat ccaaaaaaga ggaaatcatc aaaatggatg atacaaagtg gaatgcacta
3061 ggtaaagaaa ttgaggaact gaagaagaag ctacaagtat ctctggacca ctatggaaag
3121 tacaagctca aattggagag gttcctcaaa aagaagaata aaatctctaa cagcaaggat
3181 caaattaaaa agctcaccag tttgaaaaac aaattggaga gaagacaaaa tctgttgaat
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Annex II

Preferential expression of a synthetic *Plasmodium falciparum* gene with altered codons in baculovirus

By Shirley Longacre and Charles Roth

The object of the experimentation and results presented here was to test the hypothesis that genes derived from *Plasmodium falciparum* are generally not well expressed in baculovirus owing to an important divergence in codon usage between the two organisms. In this context we constructed two types of genes encoding the C-terminal portion of the Merozoite Surface Protein 1 (MSP1) of *P. falciparum* from asparagine 1613 to serine 1705. One gene was prepared by using PCR and appropriate short oligonucleotides to make a direct copy from the DNA of the Uganda-Palo Alto (FUP) strain of *P. falciparum* (Chang et al., Exp. Parasit. 67,1; 1989).. A second type of gene was constructed from five long overlapping synthetic oligonucleotides (84-87 bases) using PCR. This gene encoded the same amino acids as the first type but its codon usage was adjusted to reflect baculovirus codon preferences as determined by the analysis of baculovirus genes previously reported in the literature.

In addition to the 93 amino acids between Asn 1613 and Ser 1705 of *P. falciparum* (FUP) MSP1, each type of gene construct included sequences coding for Met 1 to Asp32 derived from *P.lasmodium vivax* (Belem strain) DNA (del Portillo et al., P.N.A.S. 88, 4043; 1991) followed by Glu-Phe derived from the EcoR1 linker used to construct the hybrid genes as detailed by Longacre et al. (Mol. Biochem. Parasit. 64, 191; 1994). The *P. vivax* derived sequence contained a signal sequence which was previously shown to function well in the baculovirus system to mediate secretion or cell surface expression of similar recombinant proteins from *P. vivax* (Longacre et al., op. cit.).

Figure 1 shows the sequence of our synthetic gene as well as the corresponding native *P. falciparum* sequence. The GC content of the native and synthetic *P. falciparum* sequences are 33% and 54% respectively. The synthetic gene contains 57/93 or 61% codon changes compared to the native gene.

Figure 2 shows immunoblots of reduced and non reduced cell lysates derived from Sf9 insect cells infected with recombinant baculovirus containing either the native or synthetic *P. falciparum* sequences. Immunoblotting was done using a pool of 13 antisera derived from *P. falciparum* hyperimmune donors. The results show that cells infected with recombinant virus containing the synthetic *P. falciparum* MSP1 sequence from each of 8 individually isolated baculovirus plaques (61-4; 71-4) show significantly more reactivity with the hyperimmune antisera than cells infected with 2 different recombinant virus isolates containing the native *P. falciparum* sequence (P1, P5). The changes in migration of the non reduced recombinant protein